ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/123.19

*Volume 123, pp. 19–29* *January–March 2013*

# Three new species of *Hymenoscyphus* from tropical China

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Abstract — Three new species of *Hymenoscyphus* collected from tropical regions of China are described and illustrated. *Hymenoscyphus magnicellulosus* possesses whitish sessile apothecia with large-celled textura prismatica at flank and isodiametric cells at the base, and inequilateral ellipsoid ascospores 12.5–19(–22) × 4.5–6.7 µm. *Hymenoscyphus uniseptatus* has white stipitate apothecia, rectangular ectal excipular cells, broadly clavate asci, and broadly fusoid 1-septate ascospores 16.7–20.5 × 5–6.5 µm. *Hymenoscyphus yunnanicus* is characterized by cream to light yellow stipitate apothecia, narrow ectal excipular hyphae, and multiguttulate fusoid ascospores 16.5–19.5 × 5–5.6 µm. Comparisons were made among the new species and their morphologically similar fungi.

Key words —*Helotiales*, morphology, taxonomy

## Introduction

*Hymenoscyphus* Gray (*Helotiaceae*) is characterized by discoid and stipitate to sessile apothecia, a white to yellowish hymenium surface, an ectal excipulum of textura prismatica or sometimes mixed with textura angularis, a medullary excipulum of textura intricata to textura porrecta, ascospores that are fusoid, ellipsoid to scutuloid, and growth on plant materials (Gray 1821; Dennis 1956; Sharma 1991; Lizoň 1992; Zhang 2002). The main characters used to distinguish species are color, shape and size of apothecia, structure of ectal excipulum, size and apical apparatus of asci, shape, size and guttulation of ascospores, and sometimes substrate specificity (Phillips 1887; Verkley 1993; White 1943; Dennis 1956; Dumont 1981a,b; Lizoň 1992).

Regional studies of *Hymenoscyphus* were carried out in Europe (Phillips 1887; Velenovský 1934; Dennis 1956, 1962, 1968; Lizoň 1992; Milekhin & Prokhorov 2008), America (Seaver 1951; Dennis 1954, 1958a, 1960; Dumont & Carpenter 1982; Gamundi 2003), Asia (Thind & Singh 1970, 1972; Thind & Sharma 1980; Sharma 1991; Zhang 2002; Han & Shin 2008), Oceania

(Dennis 1958b, 1961), and Africa (Dennis 1984; Descals et al. 1984; Fisher & Spooner 1987). More than 500 names have been connected to *Hymenoscyphus* (http://www.indexfungorum.org/Names/Names.asp), and approximately 155 species are currently accepted (Kirk et al. 2008). The genus needs a monographic treatment.

Twenty-six species are known from China (Teng 1934, 1963; Tai 1979; Zang 1983; Korf & Zhuang 1985; Zhuang & Korf 1989; Bi et al. 1990; Zhuang 1995, 1998a,b, 1999a,b, 2001, 2003a,b; Zhuang & Wang 1998a,b; Yu et al. 2000; Wang & Pei 2001; Zhang 2002; Zhang & Zhuang 2002a,b, 2004). The species previously described from China were mainly based on materials from temperate areas (Zhang 2002; Zhang & Zhuang 2002a,b, 2004), and very few species were known from tropical China.

During our examinations of the *Hymenoscyphus* collections from tropical Hainan and Yunnan provinces, we reported six species (Zheng & Zhuang 2011), of which five occur also in temperate China. As a continuation of the work, we describe and name three new species in this paper: *H. magnicellulosus*, *H. uniseptatus* and *H. yunnanicus*. Distinctions among the new species and their related fungi are discussed.

## Materials & methods

Specimens examined were collected from tropical areas of Hainan and Yunnan provinces in 1999 and 2000. Field notes were taken from apothecia in fresh condition. Dried apothecia were rehydrated with distilled water and sectioned at a thickness of 10–20 μm with a freezing microtome (YD-1508A, Yidi Medical Instrument Co., Jinhua, China). Measurements were taken from longitudinal sections and from squash mounts in lacto-phenol cotton blue solution using an Olympus BH-2 microscope (Tokyo, Japan). Iodine reactions of ascus apparatus were tested in Melzer's reagent with 3% KOH aqueous solution pretreatments. Features of apical ring were recorded according to Baral (1987). Ascospore guttulation was examined in 3% KOH aqueous solution. Photographs were taken using a Canon G5 digital camera (Tokyo, Japan) connected to a Zeiss Axioskop 2 Plus microscope (Göttingen, Germany) and a Zeiss Stemi 2000C (Göttingen, Germany) stereomicroscope. Line drawings were made using an Opton microscope (West Germany) equipped with a drawing tube. Specimens examined are deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

## Taxonomy

***Hymenoscyphus magnicellulosus*** H.D. Zheng & W.Y. Zhuang, **sp. nov.**

MycoBank MB564248 Figs 1A, 2, 5A–C

Differs from *Hymenoscyphus tamaricis* by its much smaller whitish sessile apothecia, narrower medullary hyphae, and shorter and wider asci.

Type: China, Yunnan: Pingbian, Daweishan, alt. 1900 m, on rotten bark, 4 Nov 1999, W.Y. Zhuang & Z.H. Yu 3254 (**Holotype**, HMAS 188555).

Figure 1 Gross morphology of dried apothecia on natural substrates. A. *Hymenoscyphus magnicellulosus* (holotype); B. *H. uniseptatus* (holotype); C. *H. yunnanicus* (holotype). Scale bars = 0.5 mm.

Etymology: The specific epithet refers to the large cells in ectal excipulum.

Apothecia turbinate, subsessile to sessile, scattered or 2–5 in a cluster, margin smooth, 0.6–2 mm in diam.; hymenium surface flat to convex, drying slightly to deeply concave, whitish when fresh, drying pale tan; receptacle surface smooth, paler than hymenium. Outer covering layer absent. Ectal excipulum 20–55 μm thick, of textura porrecta at margin, hyphae 3–5 μm wide; becoming textura prismatica at flank, cells 8–30 × 4–11 μm; and of textura angularis towards the base, cells up to 25 × 11 μm, or 5–15 μm in diam. if isodiametric, cells hyaline, thin- to slightly thick-walled. Medullary excipulum of textura intricata, 30–250 μm thick, hyphae hyaline, 1.5–4 μm wide, thin-walled. Subhymenium not distinguishable. Hymenium 95–150 μm thick. Asci 8-spored, 88–129 × 9–12 μm, cylindric-clavate, gradually narrowed into a short-medium stalk, base attenuate, arising from croziers, apex round to broadly papilliform, apical thickening 2–3 μm thick, J+ in Melzer's reagent with or without KOH pretreatment; apical ring weakly to moderately bluing as two lines about 1–1.5 μm long, even in width or slightly thinner upward, of the *Hymenoscyphus*-type. Ascospores 12.5–19(–22) × 4.5–6.7 μm, obliquely uniseriate to irregularly biseriate, hyaline, ellipsoid, slightly flattened on one side, occasionally 1-septate, with a dark stained area in cotton blue, containing 1–2 large oil drops and several small ones in cotton blue and KOH solution; guttules ellipsoid, rounded to irregular in shape, individuals up to 10 × 5 μm. Paraphyses filiform, slightly swelling and 2–3 μm at the apex, 1–2 μm at lower part, equal to or slightly exceeding the asci.

Additional specimens examined: China, Hainan: Changjiang, Bawangling, alt. 1150 m, on rotten wood of broad-leaved tree, 7 Dec 2000, W.Y. Zhuang, Z.H. Yu & Y.H. Zhang 3669 (HMAS 188556); Lingshui, Diaoluoshan, alt. 1080 m, on rotten wood of broad-leaved tree, 14 Dec 2000, W.Y. Zhuang, Z.H. Yu & Y.H. Zhang 3866 (HMAS 188557); Tongza, Wuzhishan, alt. 1600 m, on rotten wood of broad-leaved tree, 16 Dec 2000, W.Y. Zhuang, Z.H. Yu & Y.H. Zhang 3876 (HMAS 188558).

Notes: All examined *H. magnicellulosus* specimens share very similar macroscopic and microscopic characteristics.

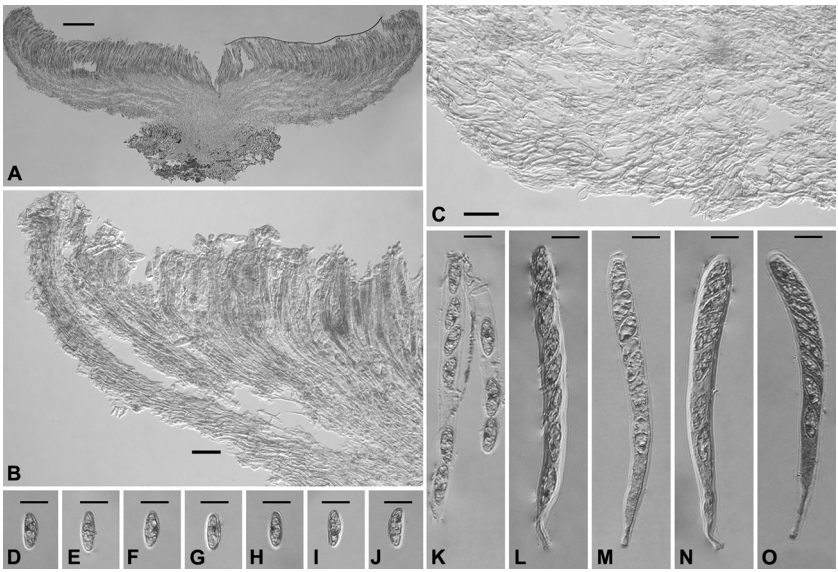


Figure 2 *Hymenoscyphus magnicellulosus* (holotype). A. Structure of apothecium; B. Excipular structure and hymenium at margin; C. Excipular structure at flank and near base; D–J. Ascospore (E. 1-septate ascospore); K. Ascospores in asci; L–O. Asci. Scale bars: A = 100 μm, B, C = 20 μm, D–O = 10 μm.

*Hymenoscyphus tamaricis* R. Galán, Baral & A. Ortega resembles *H. magnicellulosus* in lignicolous habit, ectal excipulum structure, asci arising from croziers, and guttulate and occasionally 1-septate ascospores of similar size and shape, but differs in stipitate, pale to light yellowish-ochraceous apothecia which are much larger [(1–)1.5–7(–9) mm in diam.], broader medullary hyphae (3–9 μm wide), longer and narrower asci (125–140 × 8.2–8.5 μm), and restricted only on *Tamarix* host (Galán & Baral 1997).

A species with similarly sized white apothecia and isodiametric cells in ectal excipulum is *H. immutabilis* (Fuckel) Dennis, which can be distinguished from *H. magnicellulosus* by short-stipitate apothecia, smaller and fusoid ascospores (10–13 × 4–4.5 μm) biseriate in smaller asci (80–100 × 8–9 μm), and mainly foliicolous habit (White 1943; Dennis 1956; Dumont 1981b).

*Hymenoscyphus fagineus* (Pers.) Dennis has whitish and sessile to subsessile apothecia similar to those of *H. magnicellulosus*. It is distinct in ectal excipulum composed of short rectangular cells lying at a high angle to the surface, smaller asci (65–80 × 8–9 μm) J+ and with pore strongly blue in Melzer's reagent, smaller ascospores (8–16 × 4–5 μm), and occurring only on fallen *Fagus sylvatica* pericarps (Dennis 1956; Lizoň 1992).

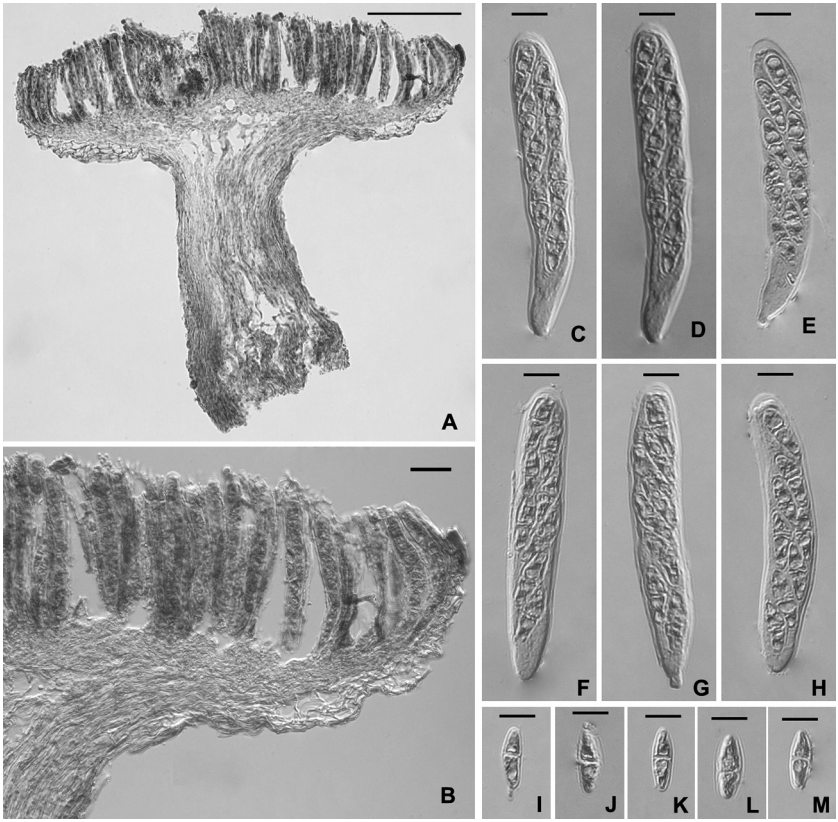


Figure 3 *Hymenoscyphus uniseptatus* (holotype). A. Structure of apothecium; B. Excipular structure and hymenium; C–H. Asci; I–M. Ascospore. Scale bars: A = 100 μm, B = 20 μm, C–M = 10 μm.

***Hymenoscyphus uniseptatus*** H.D. Zheng & W.Y. Zhuang, **sp. nov.**

MycoBank MB564250 Figs 1B, 3, 5D–F

Differs from *Hymenoscyphus jinggangensis* by its smaller apothecia, larger asci, and larger 1-septate multiguttulate ascospores.

Type: China, Yunnan: Malipo, Nanwenhe, alt. 900 m, on herbaceous stems and rotten wood of broad-leaved tree, 9 Nov 1999, W.Y. Zhuang & Z.H. Yu 3356 (**Holotype**, HMAS 188559).

Etymology: The specific epithet refers to the uniseptate ascospores.

Apothecia stipitate, scattered or 2–3 in clusters, round, margin smooth, 0.7–1 mm in diam., stipe 0.3–0.8 mm long; hymenium surface flat to discoid, white when fresh, becoming dull orange when dry; receptacle surface smooth, concolorous with hymenium. Outer covering layer absent. Ectal excipulum of textura prismatica, 15–30 μm thick, cells hyaline, slightly thick-walled,

rectangular to short rectangular, 10–22 × 7–15 μm. Medullary excipulum with two layers, outer layer of textura porrecta, 5–20 μm thick, inner layer of textura intricata, 15–30 μm thick, hyphae hyaline, 2–3 μm wide. Subhymenium not distinguishable. Hymenium 90–105 μm thick. Asci 8-spored, 80–95 × 8.5–14 μm, broadly clavate, base attenuate, arising from simple septa, apex round to conical, apical thickening 3–6 μm thick, J+ in Melzer's reagent with or without KOH pretreatment; apical ring weakly bluing as two lines about 1.5–3 μm long, equal to or becoming thinner upward, of the *Hymenoscyphus*-type. Ascospores 16.7–20.5 × 5–6.5 μm, biseriate to irregularly uniseriate, subfusoid, hyaline, 1-septate, containing 3–6 large oil drops and many small ones in cotton blue and KOH solution; guttules round or irregular in shape, individual ones up to 5 × 4 μm. Paraphyses filiform, equal to or slightly exceeding the asci, 1.5–2 μm wide.

Notes: The type specimen of *H. uniseptatus* contains apothecia growing on both herbaceous stems and rotten wood. The macroscopic and microscopic characters of apothecia on different substrates are identical. In view of this, we do not regard *H. uniseptatus* as host-specific.

*Hymenoscyphus jinggangensis* Yan H. Zhang & W.Y. Zhuang, originally described from temperate China, is most similar to *H. uniseptatus* in stipitate white apothecia, size of ectal excipulum cells, septate ascospores, and lignicolous habit. However, *H. jinggangensis* differs in larger apothecia (1–2.8 mm in diam.), thin-walled ectal excipulum cells, thicker medullary excipulum (up to 235 μm thick), smaller asci (65–83 × 7–8 μm), and smaller (9–17 × 3–5 μm) eguttulate ascospores that are 1–3(–4)-septate instead of uniseptate (Zhang 2002; Zhang & Zhuang 2002a).

Among the known *Hymenoscyphus* species, *H. musicola* (Dennis) Dennis resembles *H. uniseptatus* in stipitate, whitish apothecia, ectal excipulum of large-celled textura prismatica, 1-septate ascospores of similar size, shape and guttulation. It can be easily separated from *H. uniseptatus* by convex apothecial surface, longer asci [(90–)100–110 × 9–12 μm] arising from croziers, and occurrence on *Musa sapientum* (Dennis 1958a; Dumont 1981a).

*Hymenoscyphus varicosporoides* Tubaki is also similar in stipitate, white apothecia, and 1-septate ascospores, but differs in narrower ectal excipulum hyphae (Tubaki 1966: Pl. II fig. 2), much narrower asci (70–85 × 7–9 μm), smaller ascospores [(10–)12–15(–19) × 4–5(–7) μm] with round ends, and growth on rotting twigs in a semi-aquatic environment (Tubaki 1966).

***Hymenoscyphus yunnanicus*** H.D. Zheng & W.Y. Zhuang, **sp. nov.** Figs 1C, 4, 5G–I
MycoBank MB564251

Differs from *Hymenoscyphus crataegi* by its larger apothecia, smaller ectal excipular cells, larger asci, and larger ascospores.

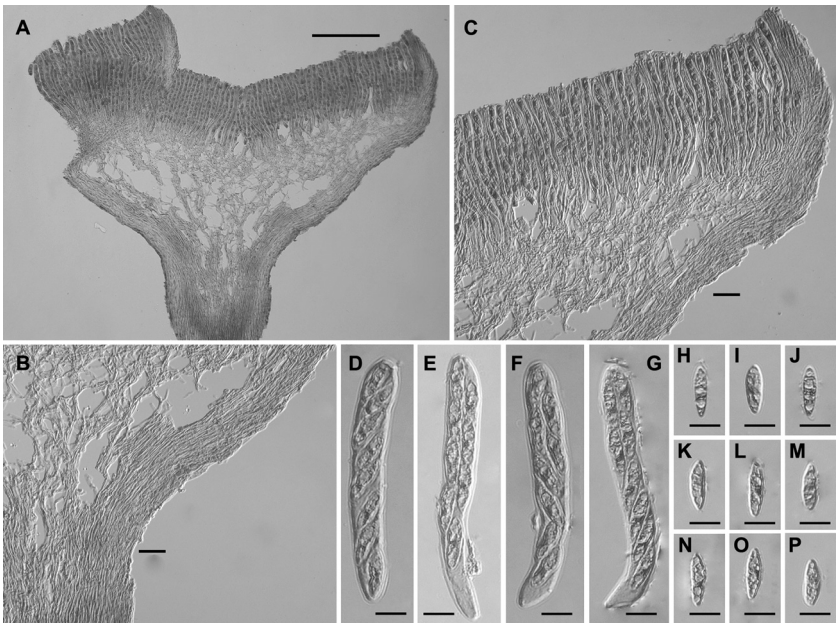


Figure 4 *Hymenoscyphus yunnanicus* (holotype). A. Structure of apothecium; B. Excipular structure and hymenium; C. Excipular structure between flank and stipe; D–G. Asci; H–P. Ascospore. Scale bars: A = 100 μm, B–C = 20 μm, D–P = 10 μm.

Type: China, Yunnan: Xichou, Xiaoqiaogou, alt. 1400 m, on rotten leaves of deciduous tree, 11 Nov 1999, W.Y. Zhuang & Z.H. Yu 3424 (**Holotype**, HMAS 188560); W.Y. Zhuang & Z.H. Yu 3430 (**Isotype**, HMAS 188561).

Etymology: The specific epithet refers to the type locality of the fungus.

Apothecia stipitate, scattered, flat to discoid, 0.6–1.5 mm in diam., stipe 0.5–0.8 mm long; hymenium surface cream to light yellow when fresh, drying brownish orange; receptacle surface smooth, paler than hymenium. Outer covering layer of 1–2 cell layers, hyphae 1–2 μm wide. Ectal excipulum of textura prismatica, 15–30 μm thick, cells hyaline, 6–12 × 3–5 μm. Medullary excipulum with two layers, outer layer of textura porrecta, 6–40 μm thick, inner layer of textura intricata, 40–110 μm thick, hyphae hyaline, 2–3 μm wide. Subhymenium not distinguishable (holotype) or 15–30 μm thick (isotype). Hymenium 95–110 μm thick. Asci (75–)87–120 × 7–11 μm, clavate, 8-spored, some with a very short stalk, base attenuate, arising from simple septa, apex round to somewhat truncate, apical thickening 1–2 μm thick, J+ in Melzer's reagent with or without KOH pretreatment; apical ring strongly bluing as two lines about 1–1.5 μm long and slightly thicker upward. Ascospores 16.5–19.5 × 5–5.6 μm, obliquely uniseriate to irregularly biseriate, hyaline, fusoid or rounded at anterior end,

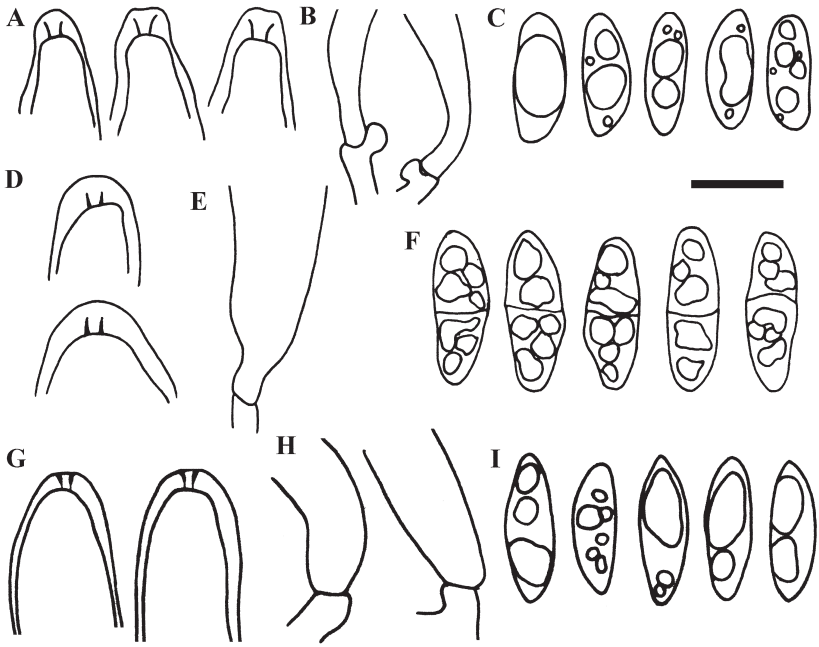


Figure 5 Ascospores and asci of *Hymenoscyphus* spp. (holotypes). A–C. *H. magnicellulosus*; D–F. *H. uniseptatus*; G–I. *H. yunnanicus*. A, D, G. Apices of asci in Melzer's reagent; B, E, H. Base of the asci showing croziers or simple septa; C, F, I. Ascospores in KOH. Scale bars = 10 μm.

slightly inequilateral, with a dark stained area in cotton blue, containing 1–3 large oil drops or multiguttulate in cotton blue and KOH solution; guttules rounded or irregular in shape, large ones 2–4 μm in diam. or in width. Paraphyses filiform, equal to or slightly exceeding the asci, 1.5–2 μm wide.

Notes: The two specimens examined are basically the same in morphology, although the subhymenium, not distinguishable in the holotype, is well developed in the isotype, which we regard as infraspecific variation.

Among the known *Hymenoscyphus* species possessing stipitate apothecia and occurring on leaves, *H. yunnanicus* is most similar to *H. phyllogenus* (Rehm) Kuntze in excipular structure. The latter differs in white and much smaller (0.3–0.5 mm in diam.) apothecia, shorter (65–75 × 8–11 μm) asci, and ascospores that are smaller (11–16 × 4–6 μm), eguttulate and ellipsoid (Dennis 1956; White 1943).

Another foliicolous *Hymenoscyphus* species that possesses stipitate apothecia and fusoid multiguttulate ascospores is *H. crataegi* Baral & R. Galán from Spain on leaves of *Crataegus monogyna*. It differs from *H. yunnanicus* by

smaller apothecia (0.4–0.6 mm in diam.), slightly gelatinized ectal excipular tissue composed of much larger cells (12–43 × 10–16 μm), smaller asci (60–80 × 6–7.5 μm), and smaller ascospores [14–17.5 × (3–)3.5–4(–4.5) μm] (Baral et al. 2006).

**Acknowledgments**

We thank Dr. Y.-Z. Wang and Dr. T. Hosoya for critically review of the manuscript and invaluable suggestions, Dr. S.R. Pennycook and Dr. L.L. Norvell for detailed corrections and valuable editorial help, Mr. H.-O. Baral for consultation, and Ms. Xia Song for making sections of some materials for this study. This work was supported by the National Natural Science Foundation of China (no. 31093440).

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